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Kinetics of Propagation of Bystander Effects in Human Cells Cultures Exposed to Low Fluences of High LET Radiations

Géraldine Gonon[1], [2], Sonia M. de Toledo[1], Michel Fromm[2] and Edouard. I. Azzam[1]

[1] Department of Radiology, New Jersey Medical School Cancer Center, Newark, NJ

[2] Laboratoire de Chimie Physique et Rayonnements Alain Chambaudet (LCPR-AC), UMR-CEA E4, Université de Franche-Comté, Besançon, France

ABSTRACT

Extensive evidence indicates that in confluent cell cultures exposed to low fluences of α particles, a high linear energy transfer (LET) radiation, the proportion of cells that express stressful effects is much higher than the number of cells irradiated. This phenomenon, termed 'the bystander effect', is now well-accepted and is thought to impact the health risks of exposure to low dose ionizing radiation. Characterization of bystander effects in cell populations exposed to high charge and high energy (HZE) particles, another type of high LET radiation, is only emerging. Here, we investigate the kinetics of propagation of signaling events that lead to induction of DNA damage in bystander cells in confluent normal human AG1522 fibroblasts exposed to a mean dose of 0.2 cGy from either 3.2 MeV α particles (LET ~ 122 keV/µm), 1 GeV/n iron ions (LET ~ 151 keV/µm), 600 MeV/n silicon ions (LET ~ 51 keV/µm) or 290 MeV/n carbon ions (LET ~ 13 keV/µm). We evaluated the formation of 53BP1 (p53 binding protein 1) foci, which localize at sites of DNA double strand breaks, as a function of time after irradiation. The fraction of cells whose nuclei were traversed by an irradiating particle was derived from Poisson statistics and estimates of cell geometry, particle fluence and energy loss. Our studies showed the following: i) The number of 53BP1 foci in control cells ranged from ~ 0.15 to 0.61 foci per cell; ii) at a mean dose of 0.2 cGy, only 1.1%, 1.4%, 3.5% and 13.4% of the cells in the exposed confluent cultures are traversed through the nucleus by α particle, iron ion, silicon ion or carbon ion tracks, respectively. Significant increases ($p<0.001$) in the fraction of cells with foci, indicative of bystander effects, were observed in cell cultures exposed to iron ions, α particles, silicon ions but not carbon ions; iii) relative to respective control, the increases in the frequency of cells with foci were detected in the first 3 h after irradiation, following which a decrease was observed. The same trend was also detected when the mean number of foci per cell was considered. Our data showed that the propagation of stressful effects from irradiated to bystander cells was dependent on the Ataxia Telangiectasia Mutated protein (ATM). The increase in foci formation over the expected value was eliminated when the cells were incubated with KU55933, a specific inhibitor of ATM.

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INTRODUCTION

The lack of clear knowledge about space radiation-induced biological effects has been singled out as the most important factor limiting the prediction of radiation risks associated with human space exploration. The expression of space radiation-induced non-targeted effects is thought to impact our understanding of the health risks that are associated with exposure to low fluences of particulate radiation encountered by astronauts during prolonged space travel.

The ionizing radiation-induced bystander effect has been broadly defined as the occurrence of biological effects in unirradiated cells as a result of exposure of other cells to radiation. Bystander effects have been extensively observed in cell cultures exposed to low fluences of α particles that target only a small fraction of the cells in the population. Changes in gene expression, induction of genetic changes including mutations and DNA damage, lethality and neoplastic transformation have been observed in bystander cells of various types suggesting that these cells contribute to the risk of exposure to ionizing radiation. The characterization of bystander effects in cell populations exposed to high charge and high energy (HZE) particles is only emerging.

One of the most serious threats to the integrity of eukaryotic genomes is DNA DSBs (Double-Strand Breaks). The 53BP1 protein rapidly localizes to discrete foci following treatment with agents that causes DNA DSBs. Here, we use this protein as a biomarker to investigate the evolution of HZE particle-induced bystander effects.

MATERIALS AND METHODS

• *Cells:* confluent, density-inhibited (95-98% in G_0/G_1) AG1522 normal human diploid fibroblasts
• *Irradiations*

	Energy (MeV/n)	LET (keV/µm)	Dose (cGy)	Φ (part./cm²)	Average hits	P(0)	P(1)	>P(1)
Iron ions	1000	151	0.2	8268	0.011	0.989	0.011	0
Alpha particles	0.8	122	0.2	10230	0.014	0.986	0.014	0
Silicon ions	600	50	0.2	24970	0.035	0.965	0.034	0.001
Carbon ions	290	13	0.2	96030	0.134	0.874	0.118	0.016

- The fluence is estimated according to the relation: $$D = 1.602.10^{-9}\frac{LET}{\rho}\times\phi$$

Where dose (D) is in *Gy* (*J.kg*$^{-1}$), LET is in *keV.µm*$^{-1}$, the fluence (φ) is in *particles.cm*$^{-2}$ and the density is (*ρ*) = *1 g.cm*$^{-3}$

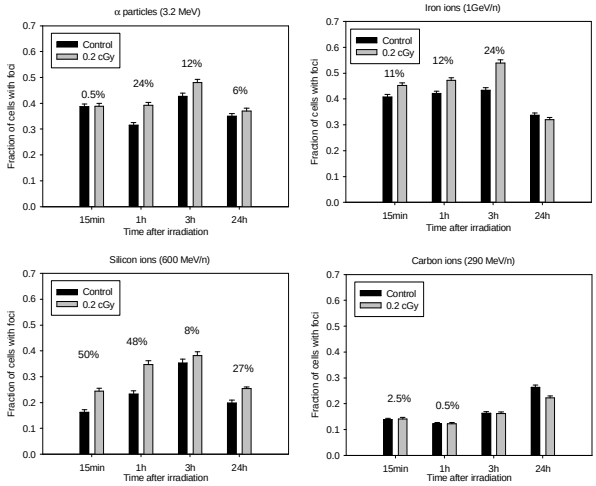
- The fluence is not uniform and is governed by Poisson distribution: $$P(N) = \frac{e^{-\bar{x}}.\bar{x}^{N}}{N!}$$

Therefore, the fraction of cells in the exposed population whose nuclei would be traversed by an iron ion or an alpha particle is ~ 1% whereas for silicon ions and carbon, it is respectively ~ 3% and ~ 12%

• *Drugs:* KU55933, a specific inhibitor of ATM
• *Fixation of cells: Following irradiation, cell cultures were held at 37°C for 15 min, 1h, 3h and 24h prior to fixation with 3.2% formaldehyde*
• *In situ immunodetection:* Anti-53BP1 antibody
• *Scoring:* For each time point of experiments, 2 irradiated dishes and 2 control dishes were analyzed manually. For each dish, more than 3000 cells were scored in 40 different fields.

Figure 1

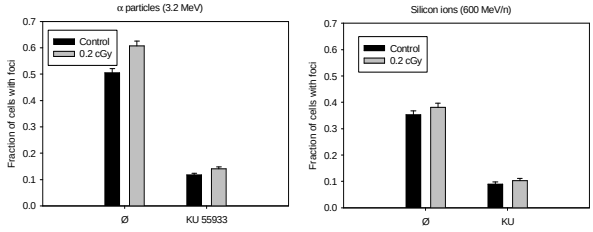
Evolution of 53BP1 foci in confluent AG1522 cell cultures as a function of time after exposure to 0.2 cGy from different energetic particles



- Data from representative experiments are shown
-The fraction of cells with foci in controls fluctuated between experiments and assay times
- Relative to respective controls, significant increases over expected values, in the fraction of cells with foci were observed in cell cultures exposed to α particles, iron ions, silicon ions but not carbon ions
-The maximum increase (% values shown above bars) in α particle- or silicon ion-irradiated cultures occurred at ~ 1 h after exposure. In iron ion-irradiated cultures, it occurred at ~ 3 h after exposure
- By 24 h after exposure, the fraction of cells with foci was attenuated
- A similar pattern of results was observed when the mean number of foci per cell was considered

Figure 2

53BP1 foci in confluent AG1522 cell cultures incubated with a specific inhibitor of ATM at 3h after exposure to 0.2 cGy from different energetic particles



- Incubation of cells with KU55933, an inhibitor of ATM, prevents the formation of 53 BP1 foci
- These data suggest that ATM kinase activity is necessary for 53BP1 foci formation

CONCLUSION

- Using 53BP1 foci formation as an endpoint, to examine, *in situ*, the stress response in cell cultures exposed to a dose by which 1% of nuclei is irradiated by a primary track of iron ions, α particles or silicon ions , stressful effects occurred in a greater number of cells than expected.
- The absence of significant increase in 53BP1 foci in cell cultures exposed to a low mean doses of carbon ions (0.2 cGy) is consistent with less complex induced DNA damage that can be readily repaired. It also suggests that energetic ions of lower LET are less efficient at inducing stressful bystander effects.
- Although nuclear fragments may have contributed to the observed stressful bystander effect, the significant increase in foci formation strongly supports the contribution to the stress response of non-targeted cells.
- Ongoing experiments are evaluating the distance of spread of the induced stress response and the underlying mechanism(s).
- Our data support the role of ATM in 53BP1 formation.
- These data may contribute to greater understanding of the health risks associated with human exposure to low level space radiation